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订货热线：400-168-3301或800-8283301
订货e-mail：order@beyotime.com
技术咨询：info@beyotime.com
网址：http://www.beyotime.com

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RIPA裂解液(强中弱套装)

产品编号	产品名称	包装
P0013E	RIPA裂解液(强中弱套装)	共150ml

产品简介：

- 碧云天生产的RIPA裂解液(RIPA Lysis Buffer)是一种传统的细胞组织快速裂解液。RIPA裂解液裂解得到的蛋白样品可以用于常规的Western、IP和ELISA等。
- RIPA的本意是Radio Immunoprecipitation Assay。RIPA裂解液的配方有很多种，根据其裂解液的强度大致可以分为强、中、弱三类。关于不同的RIPA裂解液以及碧云天生产的其它裂解液的主要特点和差异，以及如何选择裂解液可参考我们的相关网页：http://www.beyotime.com/support/lysis-buffer.htm 。
- RIPA裂解液(强中弱套装)含有 RIPA裂解液(强)、RIPA裂解液(中)和RIPA裂解液(弱)各50ml，便于实验时探索不同的实验条件。
- 用RIPA裂解液裂解得到的蛋白样品，可以用碧云天生产的BCA蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)测定蛋白浓度。由于含有较高浓度的去垢剂，不能用Bradford法测定由本裂解液裂解得到样品的蛋白浓度。

包装清单：

产品编号	产品名称	包装
P0013E-1	RIPA裂解液(强)	50ml
P0013E-2	RIPA裂解液(中)	50ml
P0013E-3	RIPA裂解液(弱)	50ml
—	说明书	1份

保存条件：

-20ºC保存，一年有效。

注意事项：

- 为取得最佳的使用效果，尽量避免过多的反复冻融。可以适当分装后使用。
- 需自备PMSF。PMSF(ST506)可以向碧云天订购。
- 裂解样品的所有步骤都需在冰上或4ºC进行。
- 关于裂解液的选择，一方面可以参考我们的相关网页：http://www.beyotime.com/support/lysis-buffer.htm 选择合适的裂解液；另一方面也需要通过一些预实验来摸索最佳的适合您实验条件的裂解液。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

使用说明：

对于培养细胞样品：

1. 融解RIPA裂解液，混匀。取适当量的裂解液，在使用前数分钟内加入PMSF，使PMSF的最终浓度为1mM。
2. **对于贴壁细胞**：去除培养液，用PBS、生理盐水或无血清培养液洗一遍(如果血清中的蛋白没有干扰，可以不洗)。按照6孔板每孔加入150-250微升裂解液的比例加入裂解液。用枪吹打数下，使裂解液和细胞充分接触。通常裂解液接触细胞1-2秒后，细胞就会被裂解。
   对于悬浮细胞：离心收集细胞，用手指把细胞用力弹散。按照6孔板每孔细胞加入150-250微升裂解液的比例加入裂解液。再用手指轻弹以充分裂解细胞。充分裂解后应没有明显的细胞沉淀。如果细胞量较多，必需分装成50-100万细胞/管，然后再裂解。
3. 充分裂解后，10000-14000g离心3-5分钟，取上清，即可进行后续的PAGE、Western和免疫沉淀等操作。
   裂解液用量说明：通常6孔板每孔细胞加入150微升裂解液已经足够，但如果细胞密度非常高可以适当加大裂解液的用量到200微升或250微升。每100万细胞用100微升RIPA裂解后获得的上清，其蛋白浓度约为2-4mg/ml，不同细胞有所不同。

对于组织样品：

1. 把组织剪切成细小的碎片。
2. 融解RIPA裂解液，混匀。取适当量的裂解液，在使用前数分钟内加入PMSF，使PMSF的最终浓度为1mM。
3. 按照每20毫克组织加入150-250微升裂解液的比例加入裂解液。(如果裂解不充分可以适当添加更多的裂解液，如果需要高

浓度的蛋白样品，可以适当减少裂解液的用量。)

4. 用玻璃匀浆器匀浆，直至充分裂解。
5. 充分裂解后，10000-14000g离心3-5分钟，取上清，即可进行后续的PAGE、Western和免疫沉淀等操作。每20mg冻存的小鼠肝脏组织用200微升RIPA裂解液裂解后获得的上清，其蛋白浓度约为15-25mg/ml，不同状态的不同组织有所不同。
6. 如果组织样品本身非常细小，可以适当剪切后直接加入裂解液裂解，通过强烈vortex使样品裂解充分。然后同样离心取上清，用于后续实验。直接裂解的优点是比较方便，不必使用匀浆器，缺点是不如使用匀浆器那样裂解得比较充分。

注：RIPA裂解液的裂解产物中经常会出现一小团透明胶状物，属正常现象。该透明胶状物为含有基因组DNA等的复合物。在不检测和基因组DNA结合特别紧密的蛋白的情况下，可以直接离心取上清用于后续实验；如果需要检测和基因组结合特别紧密的蛋白，则可以通过超声处理打碎打散该透明胶状物，随后离心取上清用于后续实验。如果检测一些常见的转录因子，例如NF-kappaB、p53等时，通常不必进行超声处理，就可以检测到这些转录因子。

附录：碧云天生产的各种裂解液主要特点、差异和选择

首先请参考下表，了解各种裂解液的主要特点和差异。

产品编号	P0013	P0013B	P0013C	P0013D	P0013F	P0013G	P0013J	P0013K
产品名称	Western及IP细胞裂解液	RIPA裂解液(强)	RIPA裂解液(中)	RIPA裂解液(弱)	NP-40裂解液	SDS裂解液	Western及IP细胞裂解液(无抑制剂)	RIPA裂解液(强，无抑制剂)
有效裂解成分	1% Triton X-100	1% Triton X-100, 1% deoxycholate, 0.1% SDS	1% NP-40, 0.5% deoxycholate, 0.1% SDS	1% NP-40, 0.25% deoxycholate	1% NP-40	1% SDS	1% Triton X-100	1% Triton X-100, 1% deoxycholate, 0.1% SDS
裂解强度	温和	强	中	温和	温和	强	温和	强
对膜蛋白的提取	一般	很好	较好	一般	一般	很好	一般	很好
对胞浆蛋白的提取	很好	很好	很好	很好	很好	很好	很好	很好
对核蛋白的提取	较好	很好	较好	较好	较好	很好	较好	很好
胞浆磷酸化蛋白提取	很好	很好	很好	很好	很好	很好	很好	很好
细胞核转录因子提取	很好	很好	很好	很好	很好	很好	很好	很好
含蛋白酶抑制剂	是	是	是	是	是	是	否	否
含磷酸酯酶抑制剂	是	是	是	是	是	是	否	否
主要用途	WB, IP, co-IP	WB, IP	WB, IP	WB, IP, co-IP	WB, IP, co-IP	WB, ChIP	WB, IP, co-IP	WB, IP

- 用于普通的Western、IP或co-IP，我们推荐使用Western及IP细胞裂解液(P0013)，该裂解液已被国内各大研究机构广泛使用，发表大量SCI论文，用户普遍反映很好。裂解细胞或组织后，没有非常粘滞的透明状DNA团块形成，不必采用超声处理等就可以非常理想地用于后续操作。另外该裂解液裂解的产物也适合用于磷酸化蛋白的Western检测。
- 对于某些特殊蛋白的IP，如果发现Western及IP细胞裂解液 (P0013)效果不是非常理想，可以尝试用RIPA裂解液(强、中或弱)或NP-40裂解液。如果发现IP的时候背景很高，即非特异的蛋白也被IP下来，则需要选用裂解强度较高的裂解液，例如RIPA裂解液(强或中)。如果发现目的蛋白无法被IP下来，则说明裂解液的强度过强，可以使用较为温和的裂解液例如RIPA裂解液(弱)或NP-40裂解液。
- 对于某些难溶解蛋白的Western，如果发现Western及IP细胞裂解液 (P0013)效果不是非常理想，可以尝试使用裂解强度更高的裂解液例如RIPA裂解液(强、中)或SDS裂解液。使用RIPA裂解液(强)的用户也非常多，发表了大量SCI论文。
- 用于特定用途需要自行添加特定抑制剂或不需要添加抑制剂时，可以考虑选购P0013J或P0013K。P0013J在很多时候可以兼容酶活性和生物小分子的检测，对于特定的酶或生物小分子的检测是否兼容需要自行测试，碧云天不提供具体的应用信息。P0013J的裂解能力比P0013K弱一些，但用于酶活性和生物小分子时，P0013J的兼容性通常会更好一些。

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